plates were allowed to dry in a laminair flow cabin. Per treatment, forty-eight 2nd stage Colorado potato beetle larvae, one in each well containing diet and bacteria, were tested. Each row of a plate (i.e. 8 wells) was considered as one replicate. The plates were kept in the insect rearing chamber at 25±2° C., 60±5% relative humidity, with a 16:8 hours light:dark photoperiod. After every 4 days, the beetles were transferred to fresh diet containing topically-applied bacteria. The beetles were assessed as alive or dead every one or three days post infestation. For the survivors, growth and development in terms of larval weight was recorded on day 7 post infestation.

[0274] For RNaseIII-deficient *E. coli* strain AB309-105, bacteria containing plasmid pGBNJ003 and those containing the empty vector pGN29 (reference to WO 00/188121A1) were tested in bioassays for CPB toxicity. Bacteria harboring the pGBNJ003 plasmid showed a clear increase in insect mortality with time, whereas little or no mortality was observed for pGN29 and diet only control (FIGS. **6***a*-LD & **7***a*-LD). The growth and development of Colorado potato beetle larval survivors, 7 days after feeding on artificial diet containing bacteria expressing dsRNA target LD010, was severely impeded (Table 10-LD, FIG. **8***a*-LD).

[0275] For *E. coli* strain BL21(DE3), bacteria containing plasmid pGBNJ003 and those containing the empty vector pGN29 were tested against the Colorado potato beetle larvae. Similar detrimental effects were observed on larvae fed diet supplemented with BL21(DE3) bacteria as for the RNAseIII-deficient strain, AB309-105 (FIGS. **6***b*-LD & **7***b*-LD). However, the number of survivors for the five clones were higher for BL21(DE3) than for AB309-105; at day 12, average mortality values were approximately 25% lower for this strain compared to the RNase III deficient strain. Also, the average weights of survivors fed on diet containing BL21(DE3) expressing dsRNA corresponding to target LD010 was severely reduced (Table 10-LD, FIG. **8***b*-LD).

[0276] The delay in growth and development of the CPB larvae fed on diet containing either of the two bacterial strains harboring plasmid pGBNJ003 was directly correlated to feeding inhibition since no frass was visible in the wells of refreshed plates from day 4 onwards when compared to bacteria harboring the empty vector pGN29 or the diet only plate. This observation was similar to that where CPB was fed on in vitro transcribed double-stranded RNA topically applied to artificial diet (see Example 3D); here, cessation of feeding occurred from day 2 onwards on treated diet.

[0277] Plant-Based Bioassays

[0278] Whole potato plants were sprayed with suspensions of chemically induced bacteria expressing dsRNA prior to feeding the plants to CPB larvae. The potato plants of variety 'line 5' were grown from tubers to the 8-12 unfolded leaf stage in a plant growth room chamber with the following conditions: 25±2° C., 60% relative humidity, 16:8 hour light: dark photoperiod. The plants were caged by placing a 500 ml plastic bottle upside down over the plant with the neck of the bottle firmly placed in the soil in a pot and the base cut open and covered with a fine nylon mesh to permit aeration, reduce condensation inside and prevent larval escape. Fifteen Colorado potato beetle larvae at the L1 stage were placed on each treated plant in the cage. Plants were treated with a suspension of *E. coli* AB309-105 harboring the pGBNJ003 plasmids (clone 1; FIG. **7***a*-LD) or pGN29 plasmid (clone 1; see FIG. **7***a*-LD). Different quantities of bacteria were applied to the plants: 66, 22, and 7 units, where one unit is defined as 10^9 bacterial cells in 1 ml of a bacterial suspension at optical density value of 1 at 600 nm wavelength. In each case, a total volume of 1.6 ml was sprayed on the plant with the aid of a vaporizer. One plant was used per treatment in this trial. The number of survivors were counted and the weight of each survivor recorded.

[0279] Spraying plants with a suspension of *E. coli* bacterial strain AB309-105 expressing target dsRNA from pGBNJ003 led to a dramatic increase in insect mortality when compared to pGN29 control. The mortality count was maintained when the amount of bacteria cell suspension was diluted 9-fold (FIG. **9**-LD). The average weights of the larval survivors at day 11 on plants sprayed with bacteria harboring the pGBNJ003 vector were approximately 10-fold less than that of pGN29 (FIG. **10**-LD). Feeding damage by CPB larvae of the potato plant sprayed with bacteria containing the pGBNJ003 plasmid was much reduced when compared to the damage incurred on a potato plant sprayed with bacteria containing the empty vector pGN29 (FIG. **11**-LD).

[0280] These experiments showed that double-stranded RNA corresponding to an insect gene target sequence produced in either wild-type or RNaseIII-deficient bacterial expression systems is toxic towards the insect in terms of substantial increases in insect mortality and growth/development delay for larval survivors. It is also clear from these experiments that an exemplification was provided for the effective protection of plants/crops from insect damage by the use of a spray of a formulation consisting of bacteria expressing double-stranded RNA corresponding to an insect gene target.

K. Testing Various Culture Suspension Densities of *Escherichia coli* Expressing dsRNA Target LD010 Against *Leptinotarsa decemlineata*

[0281] Preparation and treatment of bacterial cultures are described in Example 3J. Three-fold serial dilutions of cultures (starting from 0.25 unit equivalents) of *Escherichia coli* RNAseIII-deficient strain AB309-105 expressing double-stranded RNA of target LD010 were applied to foliages of the potato plant of variety 'Bintje' at the 8-12 unfolded leaf stage. Ten L1 larvae of the *L. decemlineata* were placed on the treated plants with one plant per treatment. Scoring for insect mortality and growth impediment was done on day 7 (i.e., 7 days post infestation).

[0282] As shown in FIG. **14**-LD, high CPB larval mortality (90 to 100%) was recorded after 1 week when insects were fed potato plants treated with a topical application by fine spray of heat-inactivated cultures of *E. coli* harboring plasmid pGBNJ003 (for target 10 dsRNA expression) at densities 0.25, 0.08 and 0.025 bacterial units. At 0.008 units, about a third of the insects were dead, however, the surviving insects were significantly smaller than those in the control groups (*E. coli* harbouring the empty vector pGN29 and water only). Feeding damage by CPB larvae of the potato plant sprayed with bacteria containing the pGBNJ003 plasmid at concentrations 0.025 or 0.008 units was much reduced when compared to the damage incurred on a potato plant sprayed with bacteria containing the empty vector pGN29 (FIG. **15**-LD).

L. Adults are Extremely Susceptible to Orally Ingested dsRNA Corresponding to Target Genes

[0283] The example provided below highlights the finding that adult insects (and not only insects of the larval stage) are extremely susceptible to orally ingested dsRNA corresponding to target genes.